AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A composition suitable ~~for use~~ to diagnose and monitor in a single step different states of tuberculosis ~~for the discrimination of~~ thus discriminating among active tuberculosis, latent infection, and recent infection with *M. tuberculosis*, said composition comprising ~~at least~~ a peptide pool of CFP-10 peptides ~~sequence~~ selected from the group of SEQ. ID No 2, No 4, No 6, and No 8 ~~and corresponding mixtures~~.

2. (Currently Amended) A composition according to claim 1, further comprising at least a ESAT-6 peptide ~~sequence~~ selected from ~~in~~ the group of SEQ. ID No 10 and No 12 and corresponding mixtures.

3. (Original) A peptide sequence selected from the group consisting of: SEQ. ID No. 2, 4, 6, 8.

4. (Currently Amended) An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis and able to discriminate ~~for the discrimination of~~ active tuberculosis, latent infection, and recent infection with *M. tuberculosis*, whereby an aliquot of whole venous blood or PBMC (peripheral blood mononuclear cells) is admixed with an effective amount of the composition according to claim 1.

5. (Previously Presented) An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, whereby an aliquot of whole venous blood or PBMC (peripheral blood mononuclear cells) is admixed with an effective amount of the composition according to claim 2.

6. (Currently Amended) An *in vitro* method ~~to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*~~ according to claim 4, said method comprising the following steps:

a) admixing an aliquot of venous blood or mononuclear cells (PBMC) isolated from venous blood with any one or more ~~a mixture comprising each~~ of the following reagents:

- Reagent 2, at least one intact protein selected in the group of ESAT-6 and CFP-10, and corresponding mixtures;
- Reagent 3: at least one ESAT-6 peptide selected in the group of SEQ ID NO 10, 12, and corresponding mixtures, diluted in a solvent
- Reagent 4: the composition according to claim 1, ~~at least one CFP-10 peptide selected in the group of SEQ ID NO 2, 4, 6, 8, and corresponding mixtures,~~ diluted in a solvent;
- Reagent 5: the composition according to claim 2 ~~a mixture of at least one ESAT-6 and CFP-10 peptides, selected in the groups of SEQ ID NO 10, 12 and SEQ ID NO 2, 4, 6, 8, and mixtures thereof,~~ diluted in a solvent;
- Reagent 6: an aspecific T-Lymphocyte stimulus, as phythoemoagglutinine (PHA), positive control;
- Reagent 7: PPD, Purified Protein Derivative; and

b) measuring T-lymphocytes response to said reagents.

7. (Canceled).

8. (Currently Amended) A method according to claim 6, wherein ~~claims 6-7 wherein the mixture~~ in step a) further aliquots of venous blood or mononuclear cells (PBMC) isolated from venous blood are admixed with ~~comprises~~:

- Reagent 1: CTR, complete culture medium or medium comprising the solvent concentration ~~present in Reagents 3-5 (negative control)~~ of the peptide pool composition as a negative control.

9. (Original) A method according to claim 8 wherein the solvent is dimethyl sulfoxide (DMSO).

10. (Currently Amended) A method according to claim 6 ~~claims 6-9~~, whereby T-lymphocytes response is measured by: ELISPOT, FACS, whole blood ELISA.

11. (Currently Amended) A method according to claim 10, wherein said T-lymphocytes response is measured as cytokine production and said cytokine is selected from the group consisting of: IFN-gamma, TNF-alpha, GMSF, and interleukins IL1-IL24.

12. (Currently Amended) A method according to claim 6, wherein the T-lymphocyte response is a $CD4^+$ T lymphocytes response ~~claims 6-11 wherein the response is mediated by CD4 T lymphocytes~~.

13. (Currently Amended) A method according to claim 6 ~~claims 6-12~~ wherein, in case whole venous blood is used, said blood is placed into heparinised test tubes, and T-lymphocyte response is assessed by ELISA on plasma.

14. (Currently Amended) A method according to claim 6 ~~claims 6-12~~ wherein, in case PBMC are used, T-lymphocyte response is assessed by ELISPOT or Flow Cytometric Analysis.

15. (Currently Amended) A method according to claim 6 ~~claims 6-12~~ wherein PBMC are obtained from whole blood by density gradient centrifugation using a method based on the use of filter-equipped tubes for separation of leukocytes.

16. (Currently Amended) A method according to claim 11, wherein T-lymphocytes response in step b) is measured after an ~~claims 6-12 wherein the~~ incubation ~~is carried out on~~ of PBMC from whole blood ~~for~~ of at least 40 hours with subsequent quantitative determination of IFN-gamma production by Antigen-Specific T lymphocytes by the ELISPOT method.

17. (Currently Amended) A method according to claim 11, wherein T-lymphocytes response in step b) is measured after an ~~claims 6-12 wherein the~~ incubation of PBMC from whole blood of ~~is carried out for~~ at least 16 hours with subsequent determination of IFN-gamma production by Antigen-Specific T lymphocytes, said determination being both qualitative in terms of presence/absence of Antigen-Specific T lymphocytes, by FACS, and quantitative in terms of percentage and frequency of specific cells per mm^3 of blood.

18. (Currently Amended) A method according to claim 11, wherein T-lymphocytes response in step b) is measured after an incubation ~~claims 6-12 wherein the incubation is performed~~ on whole blood for approximately 24 hours with subsequent quantitative determination of IFN-gamma production by Antigen-Specific T lymphocytes by ELISA.

19. (Currently Amended) A method to discriminate among: i) active tuberculosis and recent infection with *M. tuberculosis*, ii) latent infection with *M. tuberculosis*, iii) BCG vaccinated-healthy subject from T-lymphocyte responses determined according to the method of claim 6 essentially as described in table 3 ~~A method to elaborate results from output values from method according to claim 6-18, comprising the following steps~~

~~• Calculate the absolute values, from subtracting the output value of the negative control, sample admixed with Reagent 1, from the output values for the reagents R2-R7~~

~~• Compare said absolute values with the correspondent cut-off values, and if:~~

~~• below said value, the output is not valid.~~

~~• above said value, determine if it fulfils the following criteria: value for Reagents 2, 6, 7 is at least 3-fold higher than value for Reagent 1; value for Reagent 3 is at least 2-fold higher than value for Reagent 1; value for Reagents 4 and 5 is at least 4-fold higher than value for Reagent 1;~~

~~• Ascertain if the response for Reagent 6 is positive: if not, the patient is diagnosed anergic, and the assay is not further evaluable; if the response for Reagent 6 is positive :~~

~~• Ascertain if the response for Reagent 7 is positive: if not, the patient is diagnosed as a healthy subject, if so~~

~~• Ascertain if the response for Reagent 2 is positive: if not, the patient is diagnosed as BCG-vaccinated or exposed to atypical *Mycobacteria*, if so~~

~~• Ascertain if the response to Reagent 3 or 4 or 5 or a mixture of these is also positive: if not, the patient is diagnosed as a latent TB patient or a TB patient under or after efficacious anti-TB therapy; if the response to Reagent 3 or 4 or 5 or a mixture of these is positive, the patient is diagnosed as an active TB disease patient or a patient recently infected or re-infected with *M. tuberculosis*~~.

20. (Currently Amended) A method according to claim 19, wherein T-lymphocyte response is scored positive (+) when the response to:

- Reagent 2 according to claim 6 is at least 3-fold higher than that to Reagent 1 when the CTR comprises medium;
- Reagent 3 according to claim 6 is at least 2-fold higher than that to Reagent 1 when the CTR comprises the DMSO at the same concentration present in the Reagent 3;
- Reagent 4 according to claim 6 is at least 4-fold higher than that to Reagent 1 when the CTR comprises the DMSO at the same concentration present in the Reagent 4
- Reagent 5 according to claim 6 is at least 4-fold higher than Reagent 1 when the CTR comprises the DMSO at the same concentration of the Reagent 5, and
- optionally the responses to Reagents 6 and 7 according to claim 8 are measured and these are at least 3-fold higher than that to Reagent 1

~~A method according to claim 19, where the cut-off minimum is 34 SFCs reading for Reagent 3, 4 and 5 ELISPOT output, 36 SFCs reading for Reagent 2 ELISPOT output, 60 SFCs reading for Reagent 6, 7.~~

21. (Canceled).

22. (Currently Amended) A system to elaborate results from the method of claim 6, comprising ~~output values from method according to claim 6-18 characterised in that it comprises~~ means for performing the steps of the method of claim 19 ~~any of the claims from 19 to 21~~.

23. (Currently Amended) A computer program comprising computer program code means adapted to perform all the steps of claim 20 ~~claim 19-21~~ when said program is run on a computer.

24. (Currently Amended) A computer readable medium having a program recorded thereon, said computer readable medium comprising computer program code means

adapted to perform all the steps of claim 20 ~~claim 19-21~~ when said program is run on a computer.

25. (Currently Amended) A diagnostic kit to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, ~~,~~ comprising:

- Reagent 1: CTR, complete culture medium or medium comprising the solvent concentration present in Reagents 3-5;
- Reagent 2, at least one intact protein selected in the group of ESAT-6 and CFP-10, and corresponding mixtures;
- Reagent 3: ~~at least one~~ ESAT-6 peptide pool selected in the group of SEQ ID NO 10, 12, ~~and corresponding mixtures,~~ diluted in a solvent;
- Reagent 4: the composition according to claim 1 diluted in a solvent;
- Reagent 5: the composition according to claim 2 diluted in a solvent;
- Laboratory materials and instructions for test procedure.

26. (Currently Amended) A kit according to claim 25 further comprising: ~~that further comprises:~~

- Reagent 6: an aspecific T-Lymphocyte stimulus, as PHA, phythoemoagglutinine;
- Reagent 7: PPD, Purified Protein Derivative.

27. (Canceled).

28. (Canceled).

29. (Currently Amended) A diagnostic method according to claim 4, ~~Use of a kit according to claims 27-28,~~ wherein the subject to be tested is an individual selected among mammals, such as a primate, cow, sheep, pig, badger or rodent, e.g. a mouse or rat, humans being included.

30. (Currently Amended) A method according to claim 4, ~~Use of a kit according to claims 27-28~~ wherein the subjects to be tested are subjects at risk of tuberculosis.

31. (Currently Amended) A method according to claim 4, ~~Use of a kit according to claims 27-28~~ wherein the subjects to be tested are children, health care workers and immuno-compromised patients.

32. (Currently Amended) A nucleotide ~~Nucleotide~~ sequence encoding any one of the peptides according to claim 3 and selected from the group consisting of SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 5, SEQ ID NO 7.

33-36. (Canceled).